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Effects of Alcohol and Saccharin Deprivations on Concurrent Ethanol and Saccharin Operant Self-Administration by Alcohol-Preferring (P) Rats

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Abstract

Consumption of sweet solutions has been associated with a reduction in withdrawal symptoms and alcohol craving in humans. The objective of the present study was to determine the effects of EtOH and saccharin (SACC) deprivations on operant oral self-administration. P rats were allowed to lever press concurrently self-administer EtOH (15% v/v) and SACC (0.0125% g/v) for 8 weeks. Rats were then maintained on daily operant access (non-deprived), deprived of both fluids (2 weeks), deprived of SACC and given 2 ml of EtOH daily, or deprived of EtOH and given 2 ml of SACC daily. All groups were then given two weeks of daily operant access to EtOH and SACC, followed by an identical second deprivation period. P rats responded more for EtOH than SACC. All deprived groups increased responding on the EtOH lever, but not on the SACC lever. Daily consumption of 2 ml EtOH decreased the duration of the ADE. Home cage access to 2 ml SACC also decreased the ADE but to a lesser extent than access to EtOH. A second deprivation period further increased and prolonged the expression of an ADE. These results show EtOH is a more salient reinforcer than SACC. With concurrent access to EtOH and SACC, P rats do not display a saccharin deprivation effect. Depriving P rats of both EtOH and SACC had the most pronounced effect on the magnitude and duration of the ADE, suggesting that there may be some interactions between EtOH and SACC in their CNS reinforcing effects.

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Keywords

Alcohol deprivation effect; operant self-administration; alcohol-preferring P rats; repeated deprivations; Saccharin self-administration

INTRODUCTION

An association has been made between preference for sweet substances and high alcohol intake (for review, see Kampov-Polevoy et al., 1999). Sweetness-liking has been found to be correlated in humans with a paternal family history of alcoholism (Kampov-Polevoy et al., 2004). In rats, sweetness-liking and alcohol drinking have been linked by several studies. In Wistar rats, levels of saccharin drinking have been found to be positively related to later alcohol intake (Bell et al., 1994; Gosnell and Krahn, 1992). Saccharin consumption has been positively correlated with lines selectively bred for high alcohol consumption (alcohol-preferring, P; Alko Alcoholic, AA), and negatively correlated with lines selectively bred for low alcohol consumption (alcohol non-preferring, NP; Alko non-alcoholic, ANA) (Sinclair et al., 1992). Saccharin and alcohol intakes have been positively correlated in other rat lines known for their high alcohol drinking, e.g., Fawn-Hooded, Maudsley Reactive, as well as rats known for low alcohol drinking, e.g., Flinders Resistant, Flinders Sensitive, Maudsley Non-reactive (Overstreet et al., 1993).

In most rat lines studied, however, the preference for sweets tends to override the preference for EtOH. Availability of sweet substances has been shown to have an impact on the relative preference for ethanol in Fawn-hooded rats, which demonstrate an initial decrease in EtOH drinking behavior following the introduction of 0.1% saccharin (Kampov-Polevoy et al., 1995). Studies offering concurrent availability of sweetened water or a chocolate-flavored drink have shown a marked decrease in intake of EtOH, including the high-alcohol-drinking (HAD) rats (Lankford and Myers, 1994) and Sardinian alcohol-preferring (sP) rats (Colombo et al., 1997). The sP rats demonstrated suppressed acquisition and maintenance of EtOH intake with concurrent availability of 0.1 % and 1% saccharin (Colombo et al., 2005a, 2005b). In contrast, the AA rat line expresses stable levels of alcool consumption following the introduction of saccharin as an additional reinforcer (Sinclair, 1975).

When a chocolate or saccharin solution was presented as a third-choice to P rats, however, they continued to maintain a high level of EtOH self-administration, greater than 7 g/kg/day (Lankford et al., 1991). With 2-hr home cage access to 10% EtOH, the P rat has been found to consume pharmacologically relevant amounts of EtOH (Murphy et al., 1986). The P rat responds at an equal level for concurrently available 10% EtOH and 0.0125% SACC, in 2-hr alternate-day-access sessions at an FR-1 level of reinforcement (Nowak et al., 1999).

The finding that P rats drink EtOH in the presence of other palatable substances makes this selectively bred line suitable for research into experiments using concurrent access to EtOH and another palatable reinforcer. The P rat satisfies the criteria proposed as essential for an animal model of alcoholism (Cicero, 1979; Lester and Freed, 1973). This line of rats voluntarily consumes EtOH for its pharmacological effects, attains blood alcohol levels from 50 − 200 mg%, will work to obtain EtOH, and develops tolerance and dependence

through free-choice alcohol drinking (reviewed in Murphy et al. 2002; Rodd et al., 2004). McBride and Li (1998) expanded on this model, further suggesting that an animal model of alcoholism should display characteristics associated with relapse, as research has shown that the drinking patterns of human alcoholics have multiple periods of abstinence and intake (Burish et al., 1981; Hilbrom, 1990; McMillen, 1997).

Operant techniques, examining alterations in the amount of work a subject will do to obtain a reinforcement, can be used to examine the effects of repeated deprivations on the reinforcing properties of EtOH, effectively modeling relapse (Ciccocioppo et al., 2001; Hodos, 1961; Rodd et al., 2004). Specifically, the deprivation effect, a temporary increase in a particular reward-seeking behavior seen after absence of the reward, can illuminate relapse-like behavior. A saccharin deprivation effect (SDE) is seen in rats, with increasing magnitude as length of deprivation increases, suggesting that deprivation effect is a general reward phenomenon, not involved with withdrawal, or simply relegated to drugs of abuse (Neznanova et al., 2002; Wayner et al., 1972; Sinclair and Senter, 1968).

An alcohol deprivation effect (ADE) is, therefore, a voluntary, temporary increase in the intake of EtOH, as evidenced by a change in ratio of EtOH to total fluid intake following a period of deprivation (Sinclair and Senter, 1967, 1968). The ADE has been used as model of alcohol craving to study the efficacy of drugs designed to prevent relapse drinking (Heyser et al., 1998; Kornet et al., 1991; Rodd et al., 2003, 2004, 2006; Sinclair and Li, 1989; Spanagel and Zieglgansberger, 1997; Vengeliene et al., 2005).

The P rat demonstrates an ADE after a single deprivation under 24-hr free-choice drinking and 4-hr operant access conditions (McKinzie et al., 1998). In these rats, repeated cycles of alcohol availability and deprivation prolonged the expression of an ADE (Rodd et al., 2003; Rodd-Henricks et al., 2000a, 2000b). Exposure to repeated cycles of EtOH access and deprivation increases the breakpoint obtained for EtOH during a progressive ratio test (Rodd et al., 2003) and reduced the concentration of EtOH required to support self-administration directly into the posterior ventral tegmental area (Rodd et al., 2005). These results suggest that alterations in the reinforcing properties of EtOH may be taking place with repeated deprivation cycles. However, the alternate solution in these studies was water. Thus for, studies have not been conducted to determine if the presence of an alternative reinforcing compound would influence expression of an ADE.

The objective of the present study was to determine the effects of concurrent access to EtOH and SACC on expression of an ADE. The hypothesis to be tested is that an ADE and SDE would be independently expressed following prolonged abstinence of either EtOH or SACC, and that a second deprivation would increase the magnitude and duration of the ADE and SDE.

METHODS

Animals

Adult male P rats (n = 42) from the 42nd – 43rd generations weighing 250−325g at the start of the experiment were used. Rats were maintained on a 12-hr reversed light-dark cycle

(lights off at 0900 hr). Food and water were available in the home cage *ad libitum* throughout the experiment. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council 1996).

Operant Apparatus

EtOH and SACC self-administration procedures were conducted in standard two-lever experimental chambers (Coulbourn Instruments) contained within ventilated, sound-attenuated enclosures. Two operant levers, located on the same wall, were 15 cm above a grid floor and 13 cm apart. A trough was directly beneath each lever, from which a dipper cup could raise to present fluid. Upon a reinforced response on the respective lever, a small light cue was illuminated in the drinking trough and 4 seconds of dipper cup (0.1 ml) access was presented. A personal computer controlled all operant chamber functions while recording lever responses and dipper presentations.

Operant Training

Naïve P rats were placed into the operant chamber, without prior training or previous substance experience. Operant sessions were 60 min in duration and occurred daily. The EtOH concentration used for operant administration was 15% (v/v), while the SACC concentration was 0.0125% (g/v). A previous study, examining the expression of an ADE under operant conditions, utilized 15% EtOH (Rodd et al., 2003); another study determined that 0.0125% SACC was a highly palatable solution for P rats (Nowak et al., 1999).

During the initial 4 weeks of daily operant access, both solutions (water and either EtOH or SACC) were reinforced on a fixed-ratio-1 (FR-1) schedule. Previous work with P rats indicated that the acquisition of operant oral self-administration of EtOH and SACC at an FR-1 schedule occurs within the 4^{th}–6^{th} session (60-min) when these fluids are paired with water (Rodd-Henricks et al., 2002a, 2002b). At the end of this time, rats were preferentially self-administering EtOH compared to SACC (6:1 ratio). The work requirement for EtOH was increased to an FR-3 schedule at session 29, and then to FR-5 schedule at session 43, as previously described (Rodd et al, 2003). SACC requirement remained at FR-1 throughout. This concurrent FR5-FR1 for EtOH-SACC was similar to the FR5-FR1 schedule previously used for 15% EtOH-water (Rodd et al, 2003).

Repeated Cycles of Deprivation, and EtOH and/or Saccharin Access

Following 8 weeks of operant access to EtOH and SACC, rats were randomly assigned to one of four groups. One group of rats continued daily operant sessions for the duration of the experiment (non-deprived). Three deprived groups consisted of rats maintained in their home cages for two weeks (1) without access to EtOH and SACC (deprived of EtOH and SACC); (2) given daily access to 2 ml SACC but deprived of EtOH; or (3) given daily

access to 2 ml EtOH but deprived of SACC. Prior to the home-cage deprivation, P rats were, on the average, self-administering 6 ml of 15% EtOH and 1.5 ml of 0.0125 SACC. Thus, the amount received during the deprivation period was equivalent to approximately 30% of the "normal" EtOH intake and 130% of the "normal" SACC intake.

Fluid intakes were not measured in the present experiment, but it has previously been determined that under similar circumstances, non-deprived P rats consume 97% of the amount of 15% (v/v) EtOH presented by reinforcement, equivalent to 1.2 to 1.4 g/kg (Rodd et al., 2003). Body weights increased normally during the course of the experiment for all groups.

Following the initial deprivation period, P rats were given 14 consecutive daily operant sessions with both EtOH and SACC available. Subsequently, rats that were initially deprived were cycled through a second deprivation, receiving the same treatment as during the initial deprivation cycle. Non-deprived rats continued their daily operant sessions.

Statistical Analysis

Overall operant responding (60 min) data were analyzed with a mixed factorial ANOVA with a between subject factor of group and repeated measure of 'session', 'lever' and 'cycle' when applicable. The baseline measure for the factor of 'day' was the average number of responses on the EtOH lever for the 3 sessions immediately prior to deprivation. Post-hoc Tukey's b comparisons were performed to determine individual differences.

RESULTS

Acquisition and Maintenance

Within 3 sessions, P rats responded significantly more on the EtOH than SACC lever (Fig. 1). There was a significant effect of session, $F(9,369) = 38.5$; $p < 0.0001$, lever, $F(1,41) = 322.4$; $p < 0.00001$, and a session x lever interaction, $F(9,369) = 39.8$; $p < 0.0001$, during the initial 10 operant sessions. From the third session onward, P rats preferred to self-administer EtOH to the SACC solution (t-test conducted for each session, all p values < 0.002). Across the first 10 operant sessions, responding on the EtOH lever increased, $F(9,369) = 40.9$; $p < 0.0001$, with responding during sessions 4−10 being significantly greater than the average responding during sessions 1−3 (t-tests, all p values < 0.003). There was a significant effect of session on responding on the SACC lever, $F(9,369) = 11.7$; $p < 0.0001$. However, this effect was the result of responding on the SACC lever during sessions 3−5 being significantly lower than during sessions 1, 8, 9, and 10 (t-tests, p values < 0.05).

When the work requirement for EtOH increased from an FR-1 to an FR-3, there was a significant increase in the number of responses on the EtOH lever (contrasting responding from sessions 27−30; $F(3,123) = 47.4$; $p < 0.0001$. Responses on the SACC lever during this time was also altered, $F(3, 123) = 4.6$; $p = 0.004$. This effect was the result of a significant increase in responding for SACC during the initial day of FR-3 EtOH training (significantly greater responding for SACC during session 29 than during sessions 27 and 28; p values < 0.05), but there was no increase in responding during session 30 (p values > 0.12). The transient nature of this increase is also indicated by the fact that analyzing the SACC

response data between session 27− 34 indicates no significant differences across sessions, $F(7,287) = 0.4$; $p = 0.82$. Increasing the work requirement for an EtOH reinforcer from an FR-3 to an FR-5 schedule of reinforcement significantly increased the number of responses on the EtOH lever, $F(3,123) = 72.5$; $p < 0.0001$, but during this time period there was no significant effect on responding on the SACC lever, $F(3,123) = 0.6$; $p = 0.62$.

The number of reinforcements obtained mirrored the number of responses on each lever. For example, during the 3rd session, P rats on average responded 23.0 times on the EtOH lever and received 21.3 reinforcers (an average of 2 non-reinforced responses – responses during the delivery of a reinforcer were recorded but not reinforced). Lever responses made during the 4 sec presentation of a reinforcer were recorded but did not result in additional presentation of a reinforcer (non-reinforced responses). During the 3rd operant session, P rats on average responded 4.8 times on the SACC lever and received 3.5 reinforcers. On the last day of FR1 responding (session 28) P rats responded 97.9 times on the EtOH lever and received 90.4 reinforcers. During the same session, P rats responded 13.4 times on the SACC lever, but only received on average 9.6 SACC reinforcers. On the last three FR5 sessions (baseline Fig. 1, non-deprived group), P rats responded on average 303 times on the EtOH lever, and received 58 reinforcers. In contrast, during the same 3 operant sessions, P rats responded on average 16 times on the SACC lever, but received only 10 reinforcers.

EtOH and SACC Responding Following a Single Deprivation

Responding on the EtOH lever was significantly increased following a 2 week deprivation period (Fig 2, top panel; session – $F(6,228) = 15.5$; $p < 0.0001$; group × session $F(18,228) = 3.1$; $p < 0.0001$. During the first 3 re-exposure sessions, there was a significant effect of group (F values $(3,38) > 2.8$; p values < 0.05). Post-hoc comparisons indicated that during the initial 2 re-exposure sessions all deprived groups responded significantly more than non-deprived controls; during the third re-exposure session, rats deprived of both EtOH and SACC, and rats deprived of EtOH alone responded more than the non-deprived rats and the group deprived of SACC. For the group deprived of both EtOH and SACC during the first re-exposure session, the rats responded on average 533 times on the EtOH lever, and received 105 reinforcers. In contrast, these rats responded 23 times on the SACC lever, but only received 13 reinforcers.

In contrast to responses on the EtOH lever, responding on the SACC lever was not significantly altered in any of the groups following the 2 week deprivation period (Fig 2, bottom panel; session – $F(6,228) = 1.5$; $p = 0.29$; group × session $F(18,228) = 1.1$; $p = 0.33$.

EtOH and SACC Responding Following a Second Deprivation

Responding on the EtOH lever for the 3 deprived groups returned to baseline levels by the end of the 2-week re-exposure period (Fig. 3, top panel; p values > 0.35). Responses on the EtOH lever were significantly increased following a 2nd deprivation period, and were significantly higher than responses following the first deprivation (cycle – $F(1,38) = 17.4$; $p < 0.0001$; cycle × session × group $F(18,228) = 5.4$; $p < 0.001$. Overall, the magnitude of responses on the EtOH lever during the second re-exposure period was greater in rats deprived of both EtOH and SACC, or deprived of EtOH alone, compared to the first re-

exposure (increase in responding during the initial 4 and 3 re-exposure sessions, respectively; p values < 0.01). This increase in the magnitude of the responses on the EtOH lever was not observed in the SACC deprived group (p values > 0.12). Additionally, the higher responding on the EtOH lever was prolonged following the second deprivation period. In rats deprived of both EtOH and SACC, responding on the EtOH lever was increased for the initial 6 re-exposure sessions (p values < 0.008), compared to an increase in only 4 sessions following the first deprivation period (Fig. 2).

During the first 6 re-exposure sessions, there was a significant effect of group (F values (3,38) > 5.3; p values < 0.003). Post-hoc comparisons indicated that, during the initial 3 re-exposure sessions, all groups responded differently from each other, with the rats deprived of both EtOH and SACC exhibiting the highest responding of all groups. During the 4^{th} re-exposure session, responses on the EtOH lever by rats deprived of both EtOH and SACC, or deprived of EtOH alone, were significantly different from each other, and were significantly different from the other two groups. During the 5^{th} re-exposure session, rats deprived of both EtOH and SACC, or deprived of EtOH alone were different from the other two groups. During the 6^{th} re-exposure session, rats deprived of both EtOH and SACC responded more than all other groups. For the group deprived of both EtOH and SACC during the first re-exposure session, the rats responded on average 722 times on the EtOH lever, and received 142 reinforcers. In contrast, these rats responded 19 times on the SACC lever, but only received 14 reinforcers.

In contrast to responses to the EtOH lever, responding on the SACC lever was not significantly altered following the 2^{nd} deprivation period (cycle – $F(1,38) = 2.0$; $p = 0.16$; cycle × session × group $F(18,228) = 1.1$; $p = 0.36$, all other terms p values > 0.08). Responding on the SACC lever remained relatively constant (ranged between 16 ± 4 to 22 ± 6) following the second deprivation cycle.

DISCUSSION

The results of the current study indicate that in the presence of an alternate reinforcer, P rats express a robust ADE after a single deprivation, which increases in magnitude and duration with a second deprivation (Figs. 2 and 3). In addition, the present study supports previous work showing expression of an ADE by P rats (McKinzie et al., 1998; Rodd-Henricks et al., 2000a), and, furthermore, demonstrates that an ADE can be observed in the presence of SACC. Examination of data from the second deprivation cycle shows that results replicate a previous report from our lab where repeated deprivations using a single EtOH concentration in an operant paradigm results in an increase in both magnitude and duration of responding on the EtOH lever by the P rat (Rodd et al., 2003). This follows previous work that found an increase in both the magnitude and duration of expression of an ADE for P rats under 24-hr access to multiple EtOH concentrations conditions (Rodd-Henricks et al. 2001), and an increase in the duration of ADE after repeated deprivations, when a single EtOH concentration was available (Rodd-Henricks et al., 2000a).

This study shows that, when both SACC and EtOH are available concurrently in an operant setting, the P rat prefers 15% (v/v) EtOH over 0.0125% (g/v) SACC, as evidenced by the 6-

to-1 ratio of EtOH self-administration over SACC at the FR-1 schedule, as well as by a continued willingness to increase responding for EtOH proportionately as the FR requirement increased. Additionally, the ratio of obtained reinforcers: predicted reinforcers was much higher for EtOH than for SACC in P rats (e.g., prior to deprivation - EtOH ratio 96%, SACC ratio 64%). This lower concentration of SACC was chosen because it had previously been shown that in an FR-5-alternate-day access paradigm, the P rat will work to self-administer the 0.0125% SACC at a comparable level to 15% EtOH, when the second reinforcer concurrently available is water (Melendez et al., 2004; Nowak et al., 1999). While self-administration paradigms qualitatively assess the reinforcing properties of a compound, quantitative assessment of the reinforcing properties of a compound can be determine through a progressive ratio test. Recent data indicated that P rats express identical breakpoints for the self-administration of 15% EtOH and 0.0125% SACC (Toalston et al., 2007). Therefore, when tested concurrently with water, 15% EtOH and 0.0125% SACC have the same reinforcing efficacy in P rats. Additional pilot studies were conducted to establish a concentration of SACC that would produce equal responding as concurrently available 15% EtOH. To date, all concentrations of SACC tested concurrently with 15% EtOH display a similar pattern (greatly suppressed) to that observed with the 0.0125% SACC concentration (Rodd et al., unpublished findings).

The specificity of preference of EtOH over another palatable solution is currently being assessed in our laboratory. An on-going research project is examining concurrent self-administration of sucrose and EtOH in P rats. The initial data set is very intriguing and markedly different from the current data set. During the acquisition of self-administration, P rats given concurrent access to EtOH (15%) and sucrose (1−8%) self-administer significantly more EtOH and sucrose (80 and 53% more, respectively) than P rats self-administering EtOH-water or sucrose-water (Rodd et al., unpublished data). Thus, P rats given concurrent operant access to EtOH and sucrose display a ‘positive contrast’ in self-administration behaviors. Positive-contrast is a well established learning phenomenon in which animals given two reinforcers increases the self-administration of both reinforcers compared to levels of self-administration when only a single reinforcer is available (Flaherty and Largen, 1975). In addition, P rats given concurrent access to sucrose and EtOH in operant situations will self-administer concentrations of EtOH that are not self-administered when EtOH is paired with water (e.g., P rats will self-administered 45 and 60% EtOH when 2% sucrose is concurrently available). Thus the preliminary data indicate that P rats will continual to self-administer EtOH, and at a higher rate, while another caloric reinforcer is available.

All deprived groups manifested an ADE following the initial deprivation period. Rats, which had daily access to 2 ml 15% EtOH in the home cage, returned to baseline responding earlier than those without EtOH, resulting in a decrease in the duration of the initial ADE. Following the second deprivation, an increase in the duration and magnitude of the observed ADE was observed in the EtOH deprived and the EtOH plus SACC deprived groups, but not in the group deprived of SACC (rats given 2 ml of 15% EtOH in their home cages during the deprivation period). This result supports the idea that repeated alcohol deprivations may produce long-term neuronal alterations different from any changes that may occur with continued access to EtOH. This study also observed that limited home cage access to SACC

by the EtOH deprived group had a small depressive effect on EtOH responding during the re-exposure sessions, compared to responding on the EtOH lever by the EtOH plus SACC deprived group.

A potential influence of a sweetened solution (and other alternate, non-drug reinforcers) on craving behavior has previously been suggested. Taste of solutions presented, either separately or concurrently with EtOH, affects acquisition of EtOH drinking behavior (Wayner, 2002). In rhesus monkeys, both concurrent and between-session access to a 0.03% SACC solution markedly decreased responding for phencyclidine (Campbell and Carroll, 2000). Sucrose solutions given to rats prior to naloxone-precipitated withdrawal after a 6-day induction of morphine dependence decreased global withdrawal scores (Jain et al., 2004). Levels of sucrose used in this study were rather high (20−30%). A concentration of SACC higher than 0.0125% may therefore have stronger ADE-suppression effects.

Increasing the operant work requirement for access to EtOH during the study did not alter the amount of responding for SACC (approximately 20 responses/session throughout the experiment). Furthermore, following deprivations, responding for SACC remained the same. Although the data indicate that P rats will express an ADE in the presence of SACC, there was no observation of a SDE in the presence of EtOH. This suggests that EtOH, but not SACC, produced long-term CNS neuronal alterations. This idea is supported by microdialysis studies that reported increases in dopamine in the nucleus accumbens and ventral pallidum during anticipation and operant self-administration of EtOH, but not of SACC (Melendez et al., 2002; 2004). In addition, under operant conditions, P rats fail to display a SDE with higher concentration of SACC (0.025% and higher; Rodd et al., 2006 and unpublished findings).

Researchers have recently begun to address the neurobiological phenomena accompanying ADE in the P rat. In an intracranial self-administration study, a comparison between non-deprived and repeatedly deprived P rats with intra-cranial self-administration of EtOH into the posterior ventral tegmental area reported an increase in responding by the repeatedly deprived group (Rodd et al., 2005). Receptor binding assays have shown that following repeated deprivations of EtOH in inbred P (iP) rats, D1 and D2 receptor binding sites are differentially altered in the extended amygdala, accumbens, and dorsal striatum areas of the brain (Sari et al., 2006). Additional neurotransmitters systems (.i.e., glutamate and/or serotonin) probably mediate the neuroadaptions produced by exposure to a single or multiple periods of alcohol deprivation.

Overall, the results of this study indicate that an ADE effect can be observed for P rats in the presence of an alternate reinforcer, i.e., SACC, but a SDE was not observed in the presence of EtOH. In addition, because deprivation of EtOH plus SACC had a greater effect than deprivation of EtOH alone on expression of an ADE, these results suggest some overlap in the CNS circuitry mediating the reinforcing effects of EtOH and SACC.

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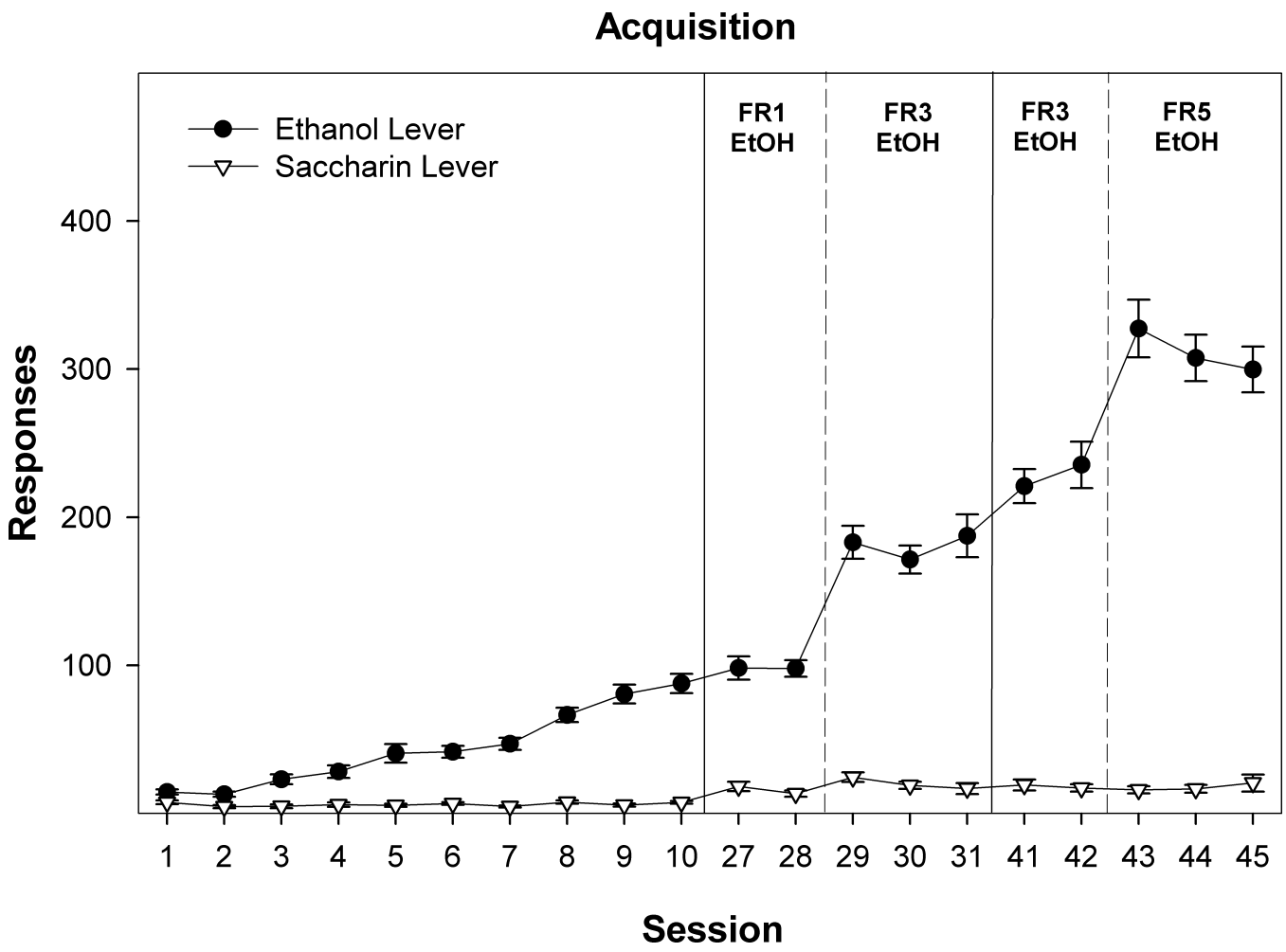

Figure 1.

Depicts the mean (± SEM) lever responses for P rats concurrently self-administering 15% EtOH and 0.0125 % saccharin (SACC). The graph also depicts the effects of increasing the work requirement for EtOH (FR1 increased to FR3, and then finally FR5) while the work requirement for SAC remained constant (FR1). As indicated in the graph, P rats preferred to self-administer EtOH from the 3rd session onward, regardless of the increase in the work requirement for EtOH (all p values < 0.002).

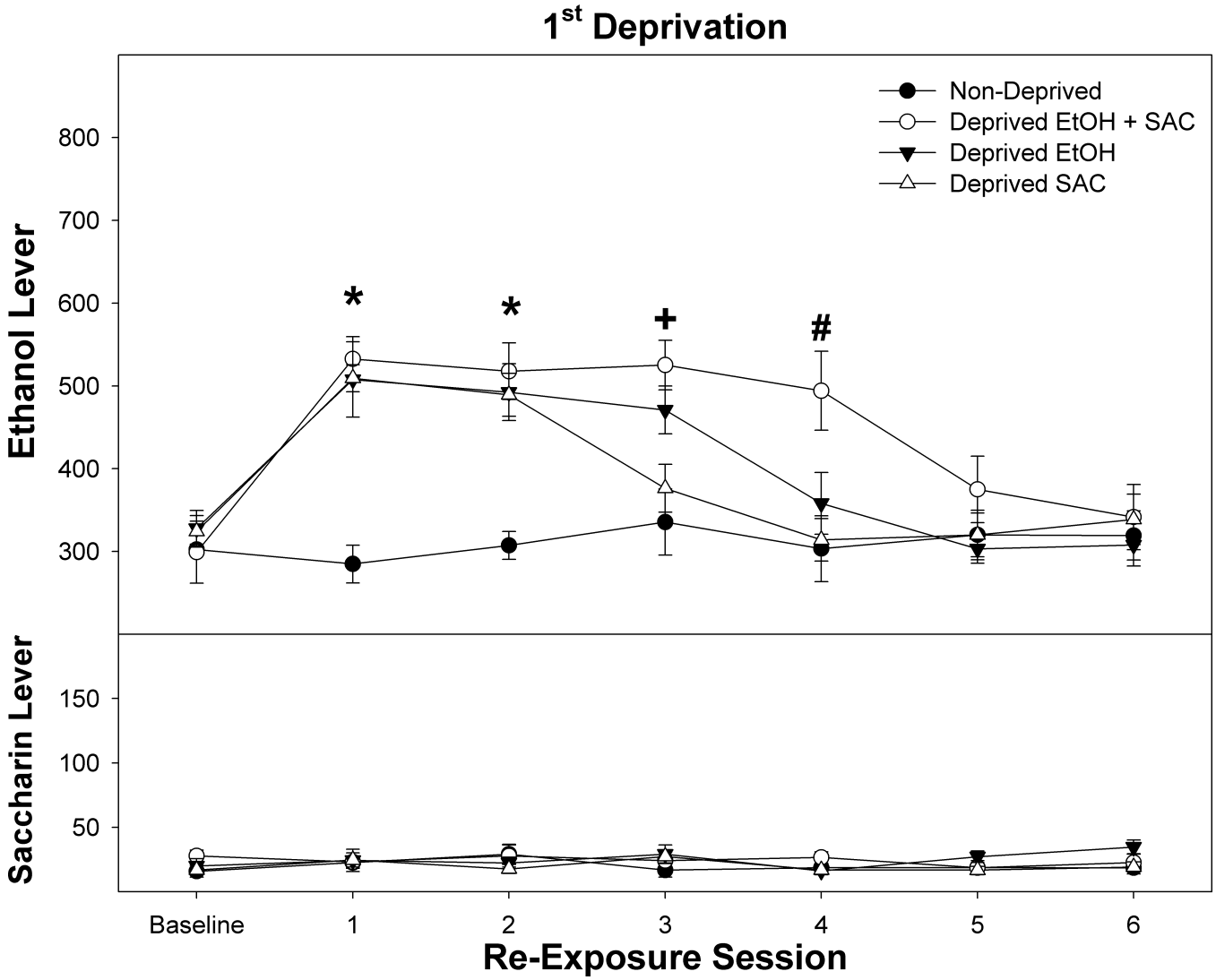

Figure 2.

Depicts the mean (± SEM) lever responses for P rats concurrently self-administering 15% EtOH (top) and 0.0125 % SACC (bottom) in rats maintained on self-administration (non-deprived) or following a 2 week deprivation period. *Asterisks* indicate that all groups are different from non-deprived and from baseline. The *Plus* symbol indicates that rats deprived of EtOH or EtOH and SACC responded significantly more than non-deprived rats and baseline. The *Pound* symbol indicates that rats deprived of EtOH and SACC responded more than all other groups and was significantly elevated compared to baseline.

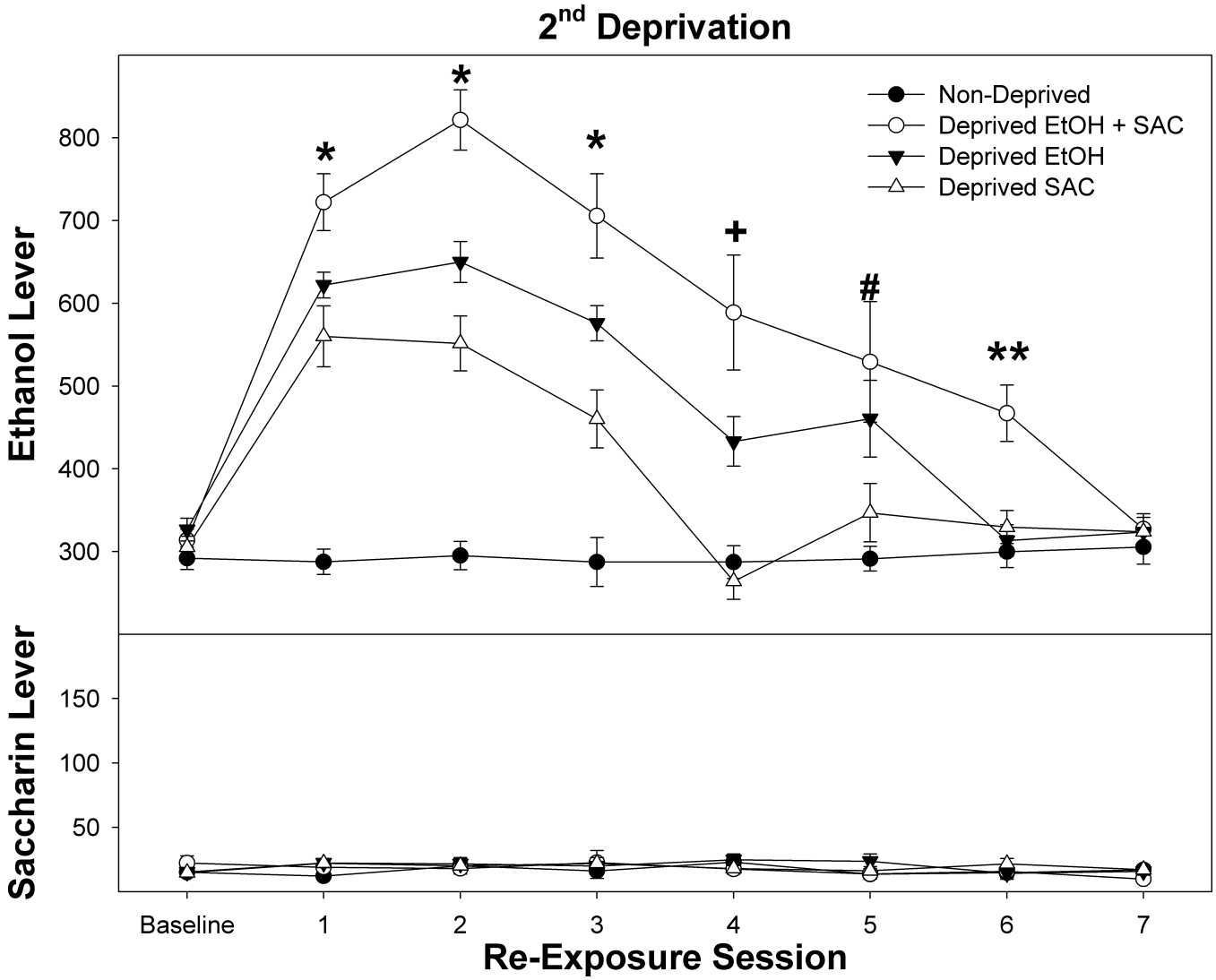

Figure 3.

Depicts the mean (± SEM) lever responses for P rats concurrently self-administering 15% EtOH (top) and 0.0125 % SACC (bottom) in rats maintained on self-administration (non-deprived) or following a 2 week deprivation period. *Asterisks* indicate that all groups are different from each other and all deprived groups are different from baseline. The *Plus* symbol indicates that rats deprived of EtOH or both EtOH and SACC were different from each other and responded significantly more than non-deprived rats and baseline. The *Pound* symbol indicates that rats deprived of EtOH and SACC responded more than all other groups and was significantly elevated compared to baseline. The *Double Asterisks* symbol indicates that rats deprived of EtOH and SACC responded significantly more than all other groups and was higher than baseline responding.